OBSERVATIONS ON MACROPHAGE BEHAVIOR IN THE FIN OF XENOPUS LARVAE

H. E. LEHMAN [1]

Zoologisches Institut, Universität Bern, and the Department of Zoology, University of North Carolina, Chapel Hill, N. C.

In a recent study by Niu and Twitty (1950) which dealt with the origin of epidermal melanophores during metamorphosis in the California salamander *Triturus torosus,* the following observations were made. Dermal melanophores which constitute the primary melanophore population during larval stages were observed to undergo autonomous disintegration shortly before metamorphosis. Phagocytic cells which gave the typical staining reactions for macrophages were observed to move into the dermis and ingest the fragments of disintegrating melanophores. Some of these cells, termed "melanophages," were subsequently seen to differentiate into typical melanophores which took their position in the epidermis and were thereafter indistinguishable from other epidermal melanophores in the skin. The conclusion to this paper contained a suggestion which was of particular interest: namely, "that the course of cellular differentiation is susceptible to control or modification in accordance with the specific character of substances transmitted or introduced through the cytoplasm" (p. 647).

The present experiments were undertaken for the purpose of examining this phenomenon in another species in which the details of cellular behavior are particularly favorable for observation. For this purpose the larvae of the clawed African toad, *Xenopus laevis* were used (see Weisz, 1945a, 1945b for normal development). In *Xenopus* larvae, the posterior third of the tail is abundantly supplied with melanophores, but the anterior ⅔ of the ventral fin is entirely free of pigment cells. In this pigment-free region, the fin is very thin and completely transparent so that the details of cell activity can be observed with great clarity.

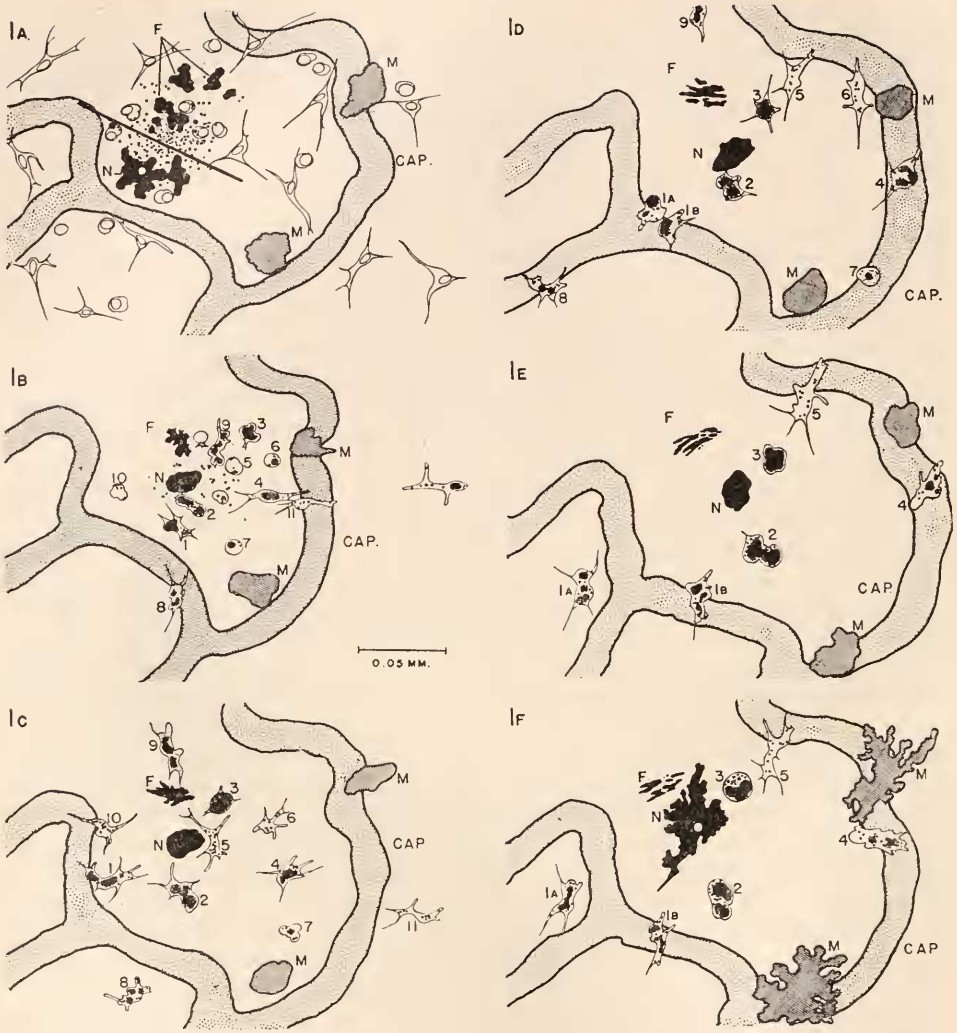
The animals used in the following study were derived from the *Xenopus* colony maintained at the Zoologisches Institut, Bern. The eggs were obtained by hormonally induced spawning following the injection of estrogenic hormones into the dorsal lymph space of mature male and female animals. The method was that of Gasche (1943) and modified by Andres *et al.* (1948). The eggs were permitted to develop until the feeding stage and thereafter the larvae were fed a suspension of nettle powder as recommended by Gasche (1943, 1944). Larvae ranging in length from 10 to 20 mm. were used in the experiments and all operations and observations were carried out on animals narcotized in one part MS 222 (Sandoz) to 7000 to

[1] National Science Foundation post-Doctoral Fellow at the Universität Bern, Sept. 1952–Sept. 1953. Now at the University of North Carolina. The writer takes this opportunity to express his deepest appreciation to Professor Fritz Baltzer for his stimulating interest and critical suggestions, to Professor F. E. Lehmann for the living material and numerous creative ideas, and to the staff of the Zoologisches Institut for their cordial hospitality and technical assistance during the tenure of the fellowship.

10,000 parts aquarium water. A special slide was constructed for the observation of the larvae and it served the dual purpose of preventing drying and at the same time elevating the fin so that high power objectives could be brought to bear on the field without endangering the thick head and trunk regions of the body. This slide consisted of a standard 1 × 3 inch slide upon which were mounted two halves of another slide, one on top of the other. They were sealed in place by deKhotinsky cement which is highly water resistant and excellent for this purpose. At one end of the mounted glass pieces was attached a "U"-shaped glass rod, 3 mm. in diameter. The latter provided a small well to hold narcotizing fluid and the head and trunk of the larva. During periods of observation, a small piece of lens paper one cm. square was placed over the head and trunk and capillarity thereafter prevented drying of the delicate epidermis. The tail of the larva extended on the elevated area of the slide and was covered by a standard 22 mm. square cover glass bearing two small drops of bee's wax at the corners placed nearest the body. This supported the cover glass at one end and permitted it to fit the tapered contour of the tail without applying pressure that would interfere with circulation.

The experimental procedures were as follows. Two cubic mm. of the hormone intermedin (2000 units vairon per cc. Intermédin, Choay Lab. Paris) from the intermediate lobe of the pituitary were injected intramuscularly at the base of the tail by means of a fine glass mouth-pipette. This hormone has the well known ability to cause melanophore expansion (Hogben and Slome, 1931; Zondek and Krohn, 1932a, 1932b; Parker, 1940). Two hours after injection, the animals with their melanophores fully expanded were narcotized and one melanophore at the edge of the pigment-free area of the ventral fin was cut into two unequal halves. One part contained the nucleus, and the other consisted of pseudopodial extensions of the cell body (N and F, Fig. 1A–F). All operations were done free-hand at 100 × magnification by means of a finely sharpened steel needle. A small notch was also made at the edge of the fin to mark the general location of the cut melanophore. A camera lucida drawing was made of the region immediately after operation; this figure showed, in addition to the cut cell, the adjacent capillaries, nerves, and melanophores so that there could be no question concerning the position of the cut cell at a later time. These cut cells were observed daily and re-drawn by camera lucida. Most cases were followed for 7 to 10 days, and a few were maintained for as long as 5½ weeks. One representative case history is given in Figures 1A–F.

Shortly after cutting, a few, and frequently many, melanin granules were released from the pigment cell. These granules were freely dispersed in the jelly matrix of the fin in the vicinity of the cell. The amputated pseudopodia tended to contract slightly during the first day following transection, and during the second day phagocytic cells usually completed the process of engulfing all except the largest cell fragments (Figs. 1A, B, C). The latter were frequently observed to remain intact for over a week. The phagocytes which participated in ingestion of melanophore fragments and pigment granules were of two varieties: 1) spherical or oval cells from the blood stream, which entered the field from adjacent capillaries, and 2) stellate mesenchyme cells *in situ* in the matrix of the fin. The former correspond to the "free macrophages" and the latter to the "fixed macrophages" referred to in the literature on the reticulo-endothelial system (see reviews by Levi, 1934; Chevremont, 1942). Preliminary experiments which in part duplicated the results of Vierling



FIGURES 1A-F. *A case history of macrophage ingestion of fragments of a cut melanophore* (camera lucida tracings). *1A*, after 24 hours; *1B*, 2 days; *1C*, 3 days; *1D*, 4 days; *1E*, 5 days, just before injection of intermedin; *1F*, 2 hours after *1E*. Abbreviations; *cap.*, capillary; *F*, non-nucleated melanophore fragment; *N*, nucleated melanophore fragment; *M*, normal melanophores; *1–11*, macrophages with ingested melanophore fragments or melanin granules.

(1926) showed that both of these cell types had a pronounced tendency to accumulate carmine injected into the fin.

The amount of melanin taken up by a single macrophage varied from a few granules (cells 5, 6, 10, 11, Figs. 1B–F), to such great quantities that the cell appeared to be gorged by the ingested particles (cells 1, 2, 3, 4 and 9). In general, those cells which contained only a moderate amount of melanin retained the particles longer than those containing excessive amounts. Some of the cells with fewer than

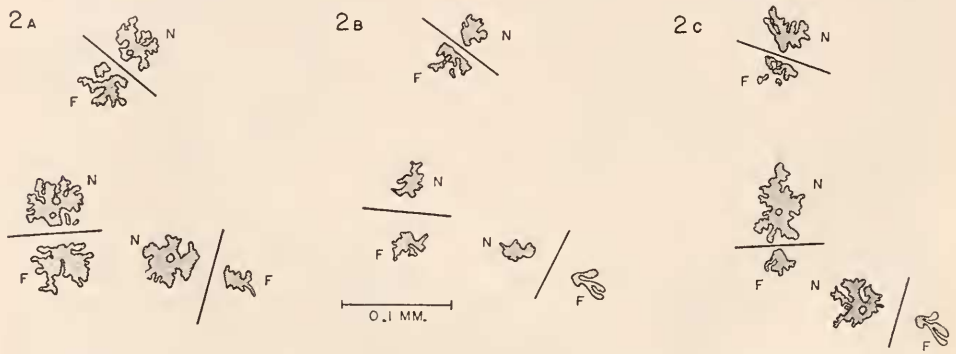
a dozen granules retained these particles for as long as 5 weeks. Macrophages containing large cell fragments usually cast off these particles into the matrix of the fin after 7 to 10 days, or, as was observed in three separate instances, the cells migrated into a capillary and were carried away by the blood stream (cells 7 and 8). The majority of macrophages containing ingested melanin usually remained in the vicinity of the cut melanophore; however, some of these cells showed marked activity (cells 6, 9, 10, and 11) and it was found to be impractical to attempt to follow cells that moved any great distance from the original field.

In none of the cases followed was there any evidence that the macrophages had acquired the ability to synthesize pigment after having phagocytized melanophore fragments. The ingested particles were generally clustered in the interior of the cells in discrete clumps which were quite different in appearance from the arrangement found in normal melanophores. It was not determined whether or not these large aggregations of melanin were contained in vacuoles; however, individual melanin grains appeared to be free in the cytoplasm. Some macrophages with moderate to large amounts of ingested material gave a superficial resemblance to contracted pigment cells (cells 2 and 3) and at first it was thought that these cells might indeed be melanophores in a true sense, since, under usual conditions, the normal melanophores remain in the contracted phase. In order to provide a decisive test to determine whether or not these cells did possess the physiological properties of melanophores, it was decided to see if they would respond in a typical manner to the influence of the hormone intermedin. To this end, four units of the hormone were injected into the base of the tail in the manner already described. Following these injections, not one of the "pseudo-melanophores" responded, whereas host melanophores became expanded maximally, as did the nucleated half of the cut melanophore (see Figs. 1E–F). Since the macrophages gave no response to the hormone, and never gave any indication that they were actively synthesizing melanin, it can be concluded that under the conditions of these experiments, at least, the ingestion of melanin did not stimulate macrophages to become melanophores. In this respect the macrophages of *Xenopus* larvae differed from these observed by Niu and Twitty (1950) in *Triturus torosus*.

It should be borne in mind that the observations of Niu and Twitty were based on normal and not on experimental conditions of behavior. Moreover, the observations on *T. torosus* were made on metamorphosing animals: a time notable for extensive developmental changes which take place under the influence of metamorphosing hormones. The latter fact makes it particularly difficult to compare the results of Niu and Twitty with those on *Xenopus* larvae. Furthermore, it was pointed out by Niu and Twitty that not all macrophages which ingested melanophore fragments in *T. torosus* became converted into melanophores; this particular feature is abundantly confirmed by the present study. Both studies therefore demonstrate that the ingestion of specific types of cellular debris does not play a constant role in determining the fate of the macrophages in question. The only histological transformation of these cells observed in the present experiments was the ready conversion of spherical "free macrophages" from the blood stream into stellate "fixed macrophages" indistinguishable from the mesenchyme cells of the fin matrix (cells 5 and 6). The reciprocal change was not so clearly demonstrable even though some of these stellate cells were observed to migrate into capillaries (cell 8). No clear-cut case

was noted in which a mesenchyme cell assumed the typical, pseudopod-free, spherical, character of those cells which originated in the blood stream. A final consideration which should be kept in mind in attempting to compare the results from *T. torosus* and *Xenopus laevis* is the importance of genetic differences which may well play a very significant part in restricting the developmental capacities and behavior properties of macrophages in the two species.

One additional observation on macrophage behavior in *Xenopus* is included at this time even though it is only indirectly related to the foregoing question of macrophage determination following ingestion of cytoplasmic particles. This concerns differences in the response of nucleated and non-nucleated melanophore fragments to the influence of the hormone intermedin. It was noted that within two or three hours after an expanded melanophore was cut, the influence of injected intermedin to a large measure disappeared (Figs. 2A–B). The nucleated half along with the great majority of host melanophores underwent a contraction of granules into a dense mass around the nucleus. The non-nucleated fragments showed less tendency



FIGURES 2A–C. The influence of intermedin on nucleated and non-nucleated fragments of melanophores (camera lucida tracings). *2A*, 3 melanophores expanded by an injection of intermedin 2 hours previously and drawn immediately after cutting (*N*, nucleated half; *F*, non-nucleated fragment); *2B*, the same cells 2 hours later, after contraction of melanin has begun and just before a second injection of intermedin; *2C*, the same cells 2 hours after the second injection of intermedin; note that only the nucleated fragments have expanded after cutting.

to contract. When intermedin was again injected into these animals two hours after cutting, the nucleated fragments and the normal cells quickly expanded, whereas the non-nucleated parts remained unchanged (Figs. 2B and 2C). These preliminary observations suggest that the cytoplasm of the melanophore requires the presence of the nucleus in order to respond to the expanding stimulus of the hormone intermedin. In any event it would appear that the ability to respond to the hormone is lost by the cytoplasm within the first four hours after separation from the nucleus.

SUMMARY

1. The hormone intermedin was injected into *Xenopus* larvae to induce pigment cell expansion. In the expanded condition melanophores were cut and macrophage ingestion of melanin granules and cell fragments was observed.

2. Macrophages retained parts of the ingested pigment cells for from one to five weeks. However, in no instance were these macrophages observed to synthesize melanin or to exhibit the physiological characteristics of melanophores as judged by their responsiveness to intermedin.

3. Preliminary observations on the influence of intermedin on nucleated and non-nucleated fragments of melanophores suggest that the cytoplasm of these pigment cells requires the presence of the nucleus in order to respond to the expanding stimulus of this hormone.

LITERATURE CITED

Andres, G., A. Bretscher, F. E. Lehmann and D. Roth, 1948. Einige Verbesserungen in der Haltung und Aufzucht von *Xenopus laevis*. *Experientia*, **5**: 1–3.

Chevremmont, M., 1942. Recherches sur l'origine, la distribution, les charactères cytologiques et les propriétés biologiques des histiocytes et des macrophages par la méthode de la culture des tissus. *Archiv. Biol.*, **53**: 281–492.

Gasche, P., 1943. Die Zucht von *Xenopus laevis* Daudin und ihre Bedeutung für die biologische Forschung. *Rev. Suisse Zool.*, **50**: 262–269.

Gasche, P., 1944. Beginn und Verlauf der Metamorphose bei *Xenopus laevis* Daud. *Helv. Physiol. Acta*, **2**: 607–626.

Hogben, L., and D. Slome, 1931. The pigmentary effector system. VI. The dual character of endocrine coordination in amphibian color change. *Proc. Roy. Soc. London, Ser. B*, **108**, 10–53.

Levi, G., 1934. Explantation, besonders die Struktur und die biologischen Eigenschaften der in vitro gezüchteten Zellen und Gewebe. *Erg. Anat. u. Entw-Gesch.*, **31**: 125–707.

Niu, M. C., and V. C. Twitty, 1950. Origin of epidermal melanophores during metamorphosis in *Triturus torosus*. *J. Exp. Zool.*, **113**: 633–647.

Parker, G. H., 1940. Neurohumors as chromatophore activators. *Proc. Amer. Acad. Arts Sci.*, **73**: 165–195.

Vierling, A., 1926. Experimenteller Beitrag zur Geschichte der Wanderzellen bei Amphibien. *Zeitschr. f. Anat. und Entwick.-Gesch.*, **81**: 448–464.

Weisz, P. B., 1945a. The development and morphology of the larva of the South African clawed toad *Xenopus laevis*. I. The third-form tadpole. *J. Morph.*, **77**: 163–192.

Weisz, P. B., 1945b. The development and morphology of the larva of the South African clawed toad, *Xenopus laevis*. II. The hatching and the first- and second-form tadpoles. *J. Morph.*, **77**: 193–217.

Zondek, B., and H. Krohn, 1932a. Hormon des Zwischenlappens der Hypophyse (Intermedin). I. Die Rotfärbung der Elritze als Testobjekt. *Klin. Woch.*, **11**: 405–408.

Zondek, B., and H. Krohn, 1932b. Hormon des Zwischenlappens der Hypophyse (Intermedin). II. Intermedin im Organismus (Hypophyse, Gehirn). *Klin. Woch.*, **11**: 849–851.